DE/ER/62691-1

Impact of Iron-Reducing Bacteria on Metals and Radionuclides Adsorbed to Humic-Coated Iron(III)Oxides

William D. Burgos, Richard F. Unz and Brian A. Dempsey – The Pennsylvania State University and Gour-Tsyh (George) Yeh – University of Central Florida

Research grant from the US Department of Energy (DOE) Natural and Accelerated Bioremediation Research (NABIR) Program, Biotransformation and Biodegradation Program Element, Grant No. DE-FG02-98ER62691, 09/15/1998 – 08/14/2002

FINAL REPORT

This is the final report for Grant No. DE-FG02-98ER62691 submitted to the DOE NABIR Program. This research has focused on (1) the role of natural organic matter (NOM), quinines, and complexants in enhancing the biological reduction of solid-phase crystalline ferric oxides, (2) the effect of heavy metals (specifically zinc) and NOM on ferric oxide bioreduction, (3) the sorption of Me(II) [Cu(II), Fe(II), Mn(II) and Zn(II)] to ferric oxides and subsequent Me(II)-promoted phase transformations of the ferric oxides, and (4) the development of reaction-based biogeochemical models to numerically simulate our experimental results.

This report is divided into four sections that list the significant findings/accomplishments, peer-reviewed papers, and student degrees associated with each of the four focus areas listed above. A final summary is also provided to highlight these technical accomplishments and describe how they contribute to the understanding and resolution of DOE issues associated with bioremediation of metals and radionuclides.

Focus Area 1 – Impact of Natural Organic Matter on Biological Iron(III) Reduction

Significant Findings/Accomplishments:

- The rate and extent of hematite bioreduction by the dissimilatory metal-reducing bacterium (DMRB) *Shewanella putrefaciens* strain CN32 was measured with eight reference humic substances, and was compared to two chemical "functional analogs" to elucidate the mechanisms by which NOM enhances solid-phase iron(III) reduction. Anthraquinone-2,6-disulfonate (AQDS) and ferrozine were used as functional analogs of electron shuttling and Fe(II) complexation, respectively.
- Fe(II) complexation and electron shuttling by NOM were equally effective mechanisms in enhancing solid-phase ferric oxide bioreduction. Previously, electron shuttling was assumed to be the sole, primary mechanism by which NOM promoted bioreduction.
- Electron shuttling stimulated the initial rate of hematite bioreduction while Fe(II) complexation increased the final extent of bioreduction.
- The enhancement of hematite bioreduction by NOM was not correlated to NOM acidity or ESR signal but most closely correlated with aromaticity/hydrophobicity.
- Hematite bioreduction slowed when the Fe(II) sorption capacity of hematite and cell surfaces was exhausted. This result suggests that solid-phase iron(III) reduction may be controlled by available hematite "free surface sites" for bacterial attachment and/or that cell vitality may be effected by Fe(II) biosorption.

DISCLAIMER

- Alternatively, hematite bioreduction may have slowed when all of the initially hydrated interfacial Fe(III) was eroded and the new sites armored by adsorbed materials, including Fe(II).
- Hematite bioreduction was mass transfer-controlled in batch systems, and Fe(II) transport away from oxide and cell surfaces appears to be the rate-limiting step.
- Hematite surface atoms are more energetic (i.e., more reducible) than "bulk" interior atoms.

Peer-Reviewed Publications:

Royer, R.A., B.H. Jeon, W.D. Burgos and B.A. Dempsey. 2003. Inhibition of Biological Reductive Dissolution of Hematite by Ferrous Iron. Environmental Science & Technology. *Submitted.*

Royer, R.A., R.F. Unz, B.A. Dempsey and W.D. Burgos. 2003. Dissimilatory Metal Reducing Bacteria in Biogeochemistry and Corrosion. Corrosion/03, Paper # 03554, NACE, Houston, TX.

Chen, J., B. Gu, R.A. Royer and W.D. Burgos. 2003. The Roles of Natural Organic Matter in Chemical and Microbial Reduction of Ferric Iron. Science of the Total Environment. *In Press.*

Royer, R.A., W.D. Burgos, A.S. Fisher, B.H. Jeon, R.F. Unz, and B.A. Dempsey. 2002. Enhancement of Hematite Bioreduction by Natural Organic Matter. Environmental Science & Technology. Volume 36: 2897-2904.

Royer, R.A., W.D. Burgos, A.S. Fisher, R.F. Unz and B.A. Dempsey. 2002. Enhancement of Biological Reduction of Hematite by Electron Shuttling and Fe(II) Complexation. Environmental Science & Technology. Volume 36: 1939-1946.

Royer, R.A. and R.F. Unz. 2002. Iron-Microbe Interactions in Corrosion and Solid Phase Mineral Dissolution. Corrosion/02, Paper # 02473, NACE, Houston, TX.

Burgos, W.D., R.A. Royer, A.S. Fisher and R.F. Unz. 2001. Enhancement of Dissimilatory Iron(III) Reduction by Natural Organic Matter. Batelle 6th International In Situ and On-Site Bioremediation Symposium - Bioaugmentation, Biobarriers, and Biogeochemistry, 6(8): 201-208, Batelle Press, Columbus OH. San Diego, CA, June 4-7, 2001.

Degrees:

Angela Fisher, Impact of Natural Organic Matter on Biological Hematite Reduction, M.S. Thesis, The Pennsylvania State University, University Park, PA, December 2000.

Focus Area 2 – Impact of Zinc and Natural Organic Matter on Biological Iron(III) Reduction

Significant Findings/Accomplishments:

- Zinc inhibited aerobic growth, nitrate bioreduction, ferric citrate bioreduction, and hematite bioreduction by *Shewanella putrefaciens* CN32. A 50% inhibitory concentration (IC_{50}) was defined as the zinc concentration that reduced electron acceptor consumption by 50% compared to the corresponding biotic no-zinc control. IC_{50} values based on free zinc for nitrate bioreduction and aerobic cell growth were essentially identical. The convergence of free zinc IC_{50} values for these two processes suggested that differences between IC_{50} values for soluble and solid-phase electron acceptors were caused by zinc sorbed to oxide and/or cell surfaces.
- Both of the NOM functional analogs, AQDS and ferrozine, increased zinc inhibition during hematite bioreduction, and increased zinc sorption.
- Contrary to our initial hypothesis, the addition of NOM (three reference humic substances tested) increased zinc inhibition during hematite bioreduction. Unlike results obtained with AQDS and ferrozine, NOM-promoted zinc inhibition occurred with or without increased zinc sorption.
- As expected, NOM decreased or eliminated zinc inhibition during nitrate bioreduction (evidence that NOM complexed Zn^{2+} and NOM-Zn(II) complexes were non-toxic). However, unexpectedly NOM caused non-toxic Mn(II) to inhibit hematite bioreduction. Taken together, these results suggest that ternary Me(II)-NOM-DMRB and/or Me(II)-NOM-hematite complexes are potent and specific inhibitors of solid-phase bioreduction.

Peer-Reviewed Publications:

Stone J.J., W.D. Burgos, R.A. Royer and B.A. Dempsey. 2003. Impact of Zinc on Biological Fe(III) and NO_3^- Reduction by *Shewanella putrefaciens* CN32. Environmental Science & Technology *Submitted.*

Stone J.J., W.D. Burgos, R.A. Royer and B.A. Dempsey. 2003. Zinc Inhibition of the Biological Reduction of Hematite by *Shewanella putrefaciens* CN32. Environmental Science & Technology *Submitted.*

Stone J.J., W.D. Burgos, R.A. Royer and B.A. Dempsey. 2003. Impact of Natural Organic Matter on Zinc Inhibition of Hematite Bioreduction by *Shewanella putrefaciens* CN32. Geochimica Cosmochimica Acta *Submitted.*

Degrees:

James Stone, The Effect of Zinc on the Biological Reduction of Hematite, Ph.D. Dissertation, The Pennsylvania State University, University Park, PA, December 2002.

Focus Area 3 – Reactions Between Metals and Ferric Oxides

Significant Findings/Accomplishments:

- Techniques were developed for the complete exclusion of O_2 from experimental systems, taking advantage of heterogeneous oxidation of Fe(II).
- The thermodynamics for the adsorption of Fe(II) on hematite and amorphous hydrous ferric oxide (HFO) were elaborated, including identification and quantification of strong sorption reactions and weak sorption reactions.
- Competitive sorption of Fe(II) and other divalent transition metals were quantified.
- A model was calibrated to describe the rate of sorption of divalent transition metals onto ferric oxides.
- The irreversible uptake of Fe(II) onto hematite, HFO, and mixtures of the two was measured and a model was constructed to explain the phenomenon. The incomplete recovery of Fe(II) had been reported by others, but for substantially higher pH and inorganic carbon concentrations.
- The transformations of HFO to other phases was studied at pH 6.8 and 8.5, and in the presence of various anions and cations. Mossbauer spectroscopy and operational extraction techniques were used to characterize the new phases.

Peer-Reviewed Publications:

Jeon, B.H., B.A. Dempsey, W.D. Burgos and R.A. Royer. 2003. Modeling the Sorption Kinetics of Divalent Metal Ions to Hematite. Water Research *Revised manuscript submitted.*

Jeon, B.H., B.A. Dempsey, W.D. Burgos and R.A. Royer. 2003. Sorption Kinetics of Fe(II), Zn(II), Co(II), Ni(II), and Cd(II) onto Hematite for Single Adsorbate and for Fe(II)/Me(II) Competitive Adsorption. Water Research *Revised manuscript submitted.*

Jeon, B.H., B.A. Dempsey and W.D. Burgos. 2003. Kinetics and Mechanisms for Reactions of Fe(II) with Fe(III) Oxides. Environmental Science & Technology *Revised manuscript submitted.*

Jang, J.H., B.A. Dempsey, G.L Catchen, and W.D. Burgos. 2003. Effects of Zn(II), Cu(II), Mn(II), NO_3^-, or SO_4^{-2} at pH 6.5 and 8.5 on Transformation of Hydrous Ferric Oxide (HFO) to More Stable Ferric Oxides. Colloids & Surfaces A *Accepted.*

Jeon, B.H., B.A. Dempsey and W.D. Burgos. 2003. A Low-Temperature Oxygen Trap for Maintenance of Strict Anaerobic Conditions. ASCE Journal of Environmental Engineering *Accepted.*

Jeon, B.H., B.A. Dempsey, W.D. Burgos and R.A. Royer. 2001. Reactions of Ferrous Iron with Hematite. Colloids & Surfaces A, 191(1-2):41-55.

Degrees:

Byong-Hun Jeon, Reactions of Ferrous Iron with Iron Oxides under an Anoxic Environment, Ph.D. Dissertation, The Pennsylvania State University, University Park, PA, December 2001.

Je-Hun Jang, Ph.D. Dissertation in progress, completion expected August 2003.

Focus Area 4 – Reaction-Based Modeling of Biological Iron(III) Reduction

Significant Findings/Accomplishments:

- Developed two computer codes for simulating reaction-based biogeochemical processes in batch systems: BIOGEOCHEM Version 1.0 and Version 2.0. Version 1.0 is a stand-alone code. Version 2.0 was designed for coupling with transport using HYDROBIOGEOCHEM. Both codes were designed for generic applications and have the following design capabilities:
  - Systematically define components,
  - Automatically remove redundant fast/equilibrium reactions,
  - Automatically remove irrelevant slow/kinetic reactions,
  - Enable rate formulation of individual reactions, one at a time, each with a measurable kinetic-variable,
  - Handle any number of species and a complete suite of mixed equilibrium and kinetic reactions,
  - Equilibrium reactions can be modeled with mass action equations or user-specified algebraic equations, and
  - Kinetic reactions can be modeled with elementary rate equations, enzymatic rate equations, n-th order rate equations, or user-specified rate equations
- Applied reaction-based models to describe the bioreduction of hematite, bioreduction of AQDS, chemical reduction of hematite by the reduced form of AQDS, Fe(II) sorption to hematite, and Fe(II) sorption to DMRB.
- Simulated the bioreduction kinetics of hematite by *Shewanella putrefaciens* CN32 in the presence of AQDS where parallel kinetic reactions lead to the production of biogenic Fe(II). From a mathematical standpoint, parallel kinetic reactions that produce the same product cannot be uniquely segregated. Therefore, separate experiments were performed to describe all important reactions using BIOGEOCHEM. This led to the first reported study where a diagonalized reaction-based model was used to simulate parallel kinetic reactions based on rate formulations/parameters independently obtained from segregated experiments.

Peer-Reviewed Publications:

- Fang, Y., G.T. Yeh and W.D. Burgos. 2003. A Generic Paradigm to Model Reaction-Based Biogeochemical Processes in Batch Systems. Water Resources Research *In press.*
- Burgos, W.D., Y. Fang, R.A. Royer, G.T. Yeh, J.J. Stone, B.H. Jeon and B.A. Dempsey. 2003. Reaction-based Modeling of Quinone-Mediated Bacterial Iron(III) Reduction. Geochimica Cosmochimica Acta *In press.*
- Burgos, W.D., R.A. Royer, Y. Fang, G. Yeh, A.S. Fisher, B.H. Jeon and B.A. Dempsey. 2002. Theoretical and Experimental Considerations Related to Reaction-Based Modeling: A Case Study Using Fe(III) Oxide Bioreduction. Geomicrobiology Journal. Volume 19: 253-287.
- Yeh, G.T., W.D. Burgos and J.M. Zachara. 2001. Modeling and measuring biogeochemical reactions: System consistency, data needs, and rate formulation. Advances in Environmental Research. 5:219-237.

Peer-Reviewed Proceedings:

- Yeh, G.T., Y.L. Fang and W.D. Burgos. 2002. A New Paradigm of Reaction-based Water Quality Modeling. Proc. Fifth International Conference on Hydroinformatics. Volume One - Model Development and Data Management p. 488-493. Cardiff, U.K, July 1-5, 2002.
- Suk, H. and G.T. Yeh. 2002. Particle tracking algorithm for the Lagrangian-Eulerian finite element method under the transient conditions in multi-dimensions. Proc. XIV International Conference on Computational Methods in Water Resources Vol. 2 p.1035-1042. Delft, The Netherlands, June 23-28, 2002.
- Suk, H. and G. T. Yeh. 2002. Multiphase flow simulations in fractional flow approach with general boundary condition considering phase configuration change in the system. Proc. XIV International Conference on Computational Methods in Water Resources Vol. 1 p. 257-264. Delft, The Netherlands, June 23-28, 2002.
- Fang, Y. and G.T. Yeh. 2002. Numerical modeling of reactive chemical transport under multiphase systems. Proc. XIV International Conference on Computational Methods in Water Resources Vol. 1 p. 671-678. Delft, The Netherlands, June 23-28, 2002.
- Yeh, G.T., W.D. Burgos and Y.L. Fang. 2000. Modeling biogeochemical kinetics: Issues and data needs. In Computational Methods in Water Resources Volume 1 Computational Methods for Subsurface Flow and Transport. p. 435-442. A.A. Balkema, Rotterdam, Netherlands.

Technical Reports:

- Yeh, G.T., Y.L. Fang and W.D. Burgos. 2003. BIOGEOCHEM 1.0: A Numerical Model to Simulate BIOGEOCHEMical Reactions under Multiple Phase Systems. Technical Report. Dept. of Civil and Environmental Engineering, University of Central Florida, Orlando, FL 32816
- Yeh, G.T., Y.L. Fang and W.D. Burgos. 2003. BIOGEOCHEM 2.0: A Numerical Model to Simulate BIOGEOCHEMical Reactions under Multiple Phase Systems. Technical Report. Dept. of Civil and Environmental Engineering, University of Central Florida, Orlando, FL 32816
- Yeh, G.T., Y.L. Fang and W.D. Burgos. 2003. HYDROBIOGEOCHEM 2.0: A Numerical Model of Reactive HYDROBIOGEOCHEMical Transport under Multiple Phase Flows and Nonisothermal Conditions. Technical Report. Dept. of Civil and Environmental Engineering, University of Central Florida. Orlando, FL 32816

Degrees:

Yilin Fang, Reactive Chemical Transport Under Multiphase System, Ph.D. Dissertation, The Pennsylvania State University, University Park, PA, May 2003.

Heejun Suk, Development of 2- and 3-D Simulator for Three-Phase Flow with General Initial and Boundary Conditions on the Fractional Flow Approach, Ph.D. Dissertation, The Pennsylvania State University, University Park, PA, December 2002.

Project Summary

The stimulation of dissimilatory iron reduction has been proposed for the bioremediation of reducible metals [e.g., Cr(VI)] and radionuclides [e.g., Tc(VII), U(VI)]. The addition of NOM along with the electron donors and nutrients selected to stimulate DMRB activity has also been proposed to possibly enhance performance of this bioremediation strategy. Results from this study have furthered our understanding of how NOM enhances biological iron reduction, and demonstrated for the first time that NOM may interact with divalent cations to inhibit biological iron reduction. This would suggest that other side reactions with NOM addition (e.g., NOM complexation of metal co-contaminants, facilitated transport of contaminant-NOM complexes) must also be considered before field implementation.

Results from this study have also furthered our understanding of the complex interactions between metal contaminants and solid-phase ferric oxides (e.g., sorption, co-precipitation, sorption-induced phase transformations). These interactions are important because they directly affect the bioavailability and bioenergetics associated with biological iron reduction, and (directly or indirectly) the fate of associated metal contaminants.

A new generation reaction-based model was developed in this study. This model (BIOGEOCHEM) was used to test proposed kinetic rate formulations and estimate associated rate parameters based on experimental results. Most importantly, we demonstrated that parallel kinetic reactions could be resolved by the careful design of separate experiments to independently parameterize important reactions. These results suggest that complex biogeochemical systems which contain parallel kinetic reactions can be simulated using reaction-based models provided experiments are conducted to incrementally increase system complexity.

Results from this study led to several peer-reviewed publications, conference proceedings and presentations, technical reports, and to the completion of several graduate student degrees. Specifically, this study will lead to 20 peer-reviewed publications (nine published, two in-press, two accepted, seven submitted), over 10 conference proceedings, over 20 conference presentations (both oral and posters), five Ph.D. degrees, and one M.S. degree (all degrees conferred in Civil and Environmental Engineering).